

Attorney Docket Number 54763-8001US02



EXHIBIT B

PAGES FROM

LABORATORY NOTEBOOK

17913038
100% v/v HCl
100% H2SO4



Drug sensitivity and growth curves.

JAN 07 2003

Cisplatin test.

6 wells per line \times 2 \rightarrow 12 wells
for duplicates.

Concentrations:

0	0
0.1	μM
0.5	"
2.0	"
10.0	"
50.0	"

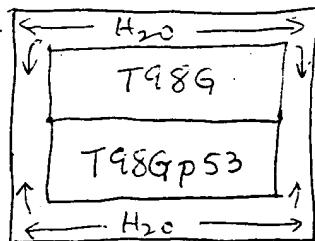


plate 2×10^4 cells
per well, settle
overnight.

treat 1-hour w/ cisplatin
1 hr. 15 min.

Count cells on

Growth curve

6 wells per line \times 2 \rightarrow 12 wells.

2000 cells per well.

T98Gp53 (culture previously assayed on G418 since) Kept without

1 almost confluent 6 cm dish
trypsinized and resuspended in 5 ml.

$$\frac{103 \text{ cells}}{9 \text{ squares}} = 11 \times 10^4 \text{ per ml.}$$

11,000 / 100 μ l.

22,000 / 200 μ l. \leftarrow per well. 48 well dish.

for drug test, add 200 μ l per well + 500 μ l medium.

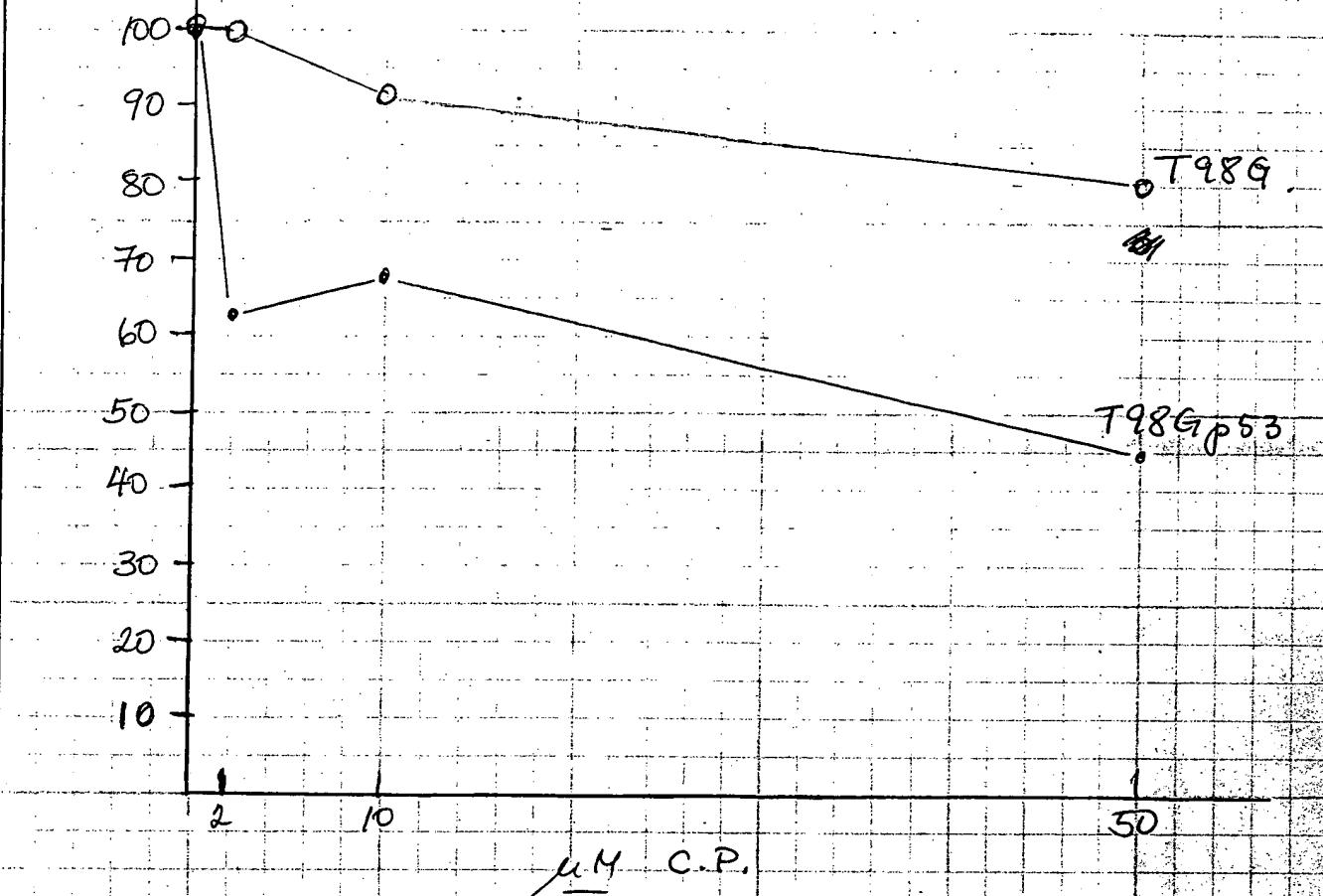
for growth curve, plate 1500 cells per well.

11,000 cells / 100 μ l.
1500 cells / 14 ml.

196 μ l (14 wells) into 6.8 ml. \rightarrow 7 ml total,
0.5 ml per well.



Cisplatin sensitivity



cells plated in 48 well plates, 2 wells per point, at 20,000 cells per well (very dense).

cells treated 1 hr $\mu\text{g}/\text{cp}$ medium changed, and cell counts were made one week later.

plating
treatment
counts

500 + 9 kcal

IRUS 006 15743072



Cisplatin test. T98G, T98G p53. (grown w/o 6418)

Stock cisplatin in DMEM at $4 \mu\text{M}$.

treatment done in 48-well plates, 0.5 ml per well.
110,000 cells per well plated out on previous day.

prepare 2 mls each concentration:

$10 \mu\text{M}$	add 5ul stock	($5 \mu\text{l} \cdot 4 \mu\text{M}$ in 2 mls.)
$20 \mu\text{M}$	10ul "	
30	" 15ul.	
40	20	
60	30	
120	60	
240	120	

following 1 hr. treatment, trypsin cells w/ 100ul trypsin, add 40ul complete medium; pipet up and down w/ 100ul tip to disperse, then replace in duplicate

and/or 5ul \rightarrow 2 plates (~ 100 colonies)
and/or 100ul \rightarrow 2 plates (~ 2000 colonies).

as follows:

control (0)	100 ($\times 2$ plates)	2000 ($\times 2$ plates)
$10 \mu\text{M}$	"	"
$20 \mu\text{M}$	"	"
$30 \mu\text{M}$	"	"
$40 \mu\text{M}$	"	"
$60 \mu\text{M}$	"	"
$120 \mu\text{M}$	"	"
$240 \mu\text{M}$	-	"
	8 plates	16 plates
	24 plates per line.	

The remaining cells in 48 well dish were allowed to continue to grow and were counted on

1/18/14
ABINGDON

T984 PC³

$$10\mu\text{H} \quad 68 \times 4 \times 10^4 / \text{mole}$$

60
~~60~~

48
~~48~~

66

~~66~~

#

$$120\mu\text{H} \quad (1\frac{1}{9})$$

(0.4)
(0.4)

240 μH

1/9

(0.4)

20 μH 53

56
(130)

64

0 41

30 μH

24

(96)

22

26

% max # cells.

10 μH

100%

20 μH

95%

30 μH

40%

40 μH

10%

0.5%

0.17%

60 μH

0.17%

120 μH

0.17%

240 μH

0.17%

$$\frac{1}{T_{\text{eff}}^2} = \frac{88}{83} x 4 + 10^4 \text{ cm}^{-2}$$

40/14

88

(364)

१०८

$$85 \times 4 \times 10^4 / \text{me.}$$

60 μm

三

10μg 10³
115 (429)
110

11/11/11

(429) 103

25
21
61

8

20%

20μg

~~10~~ 58

(365) 88
379 91

120 μs

6/4

1.8 10

2.4%

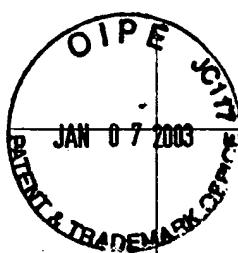
30μH

85 95

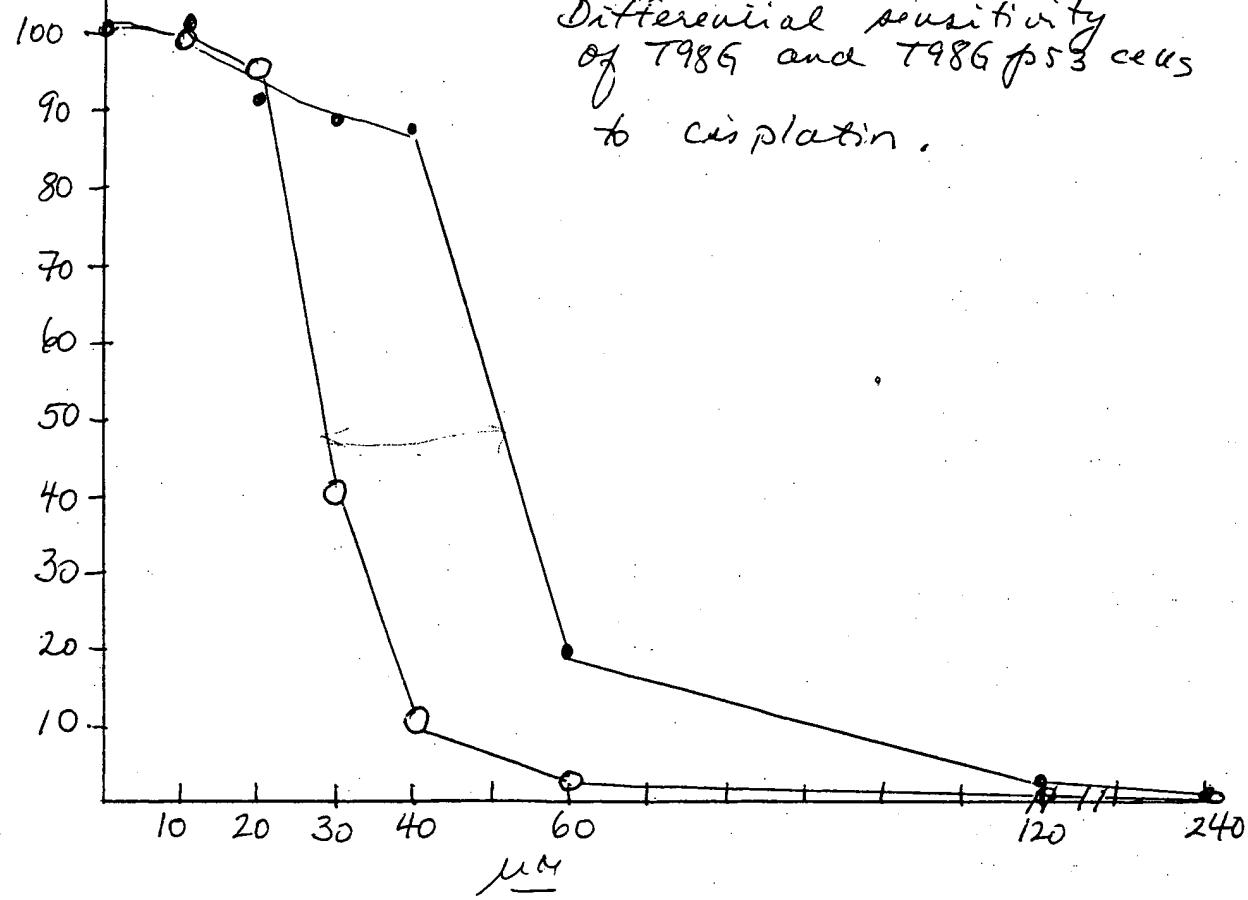
101

89

~~tb~~



42-381 50 SHEETS 5 SQUARE
42-382 100 SHEETS 5 SQUARE
42-389 200 SHEETS 5 SQUARE
NATIONAL



E.C.50 $\sim 27 \mu\text{M}$ T98G p53

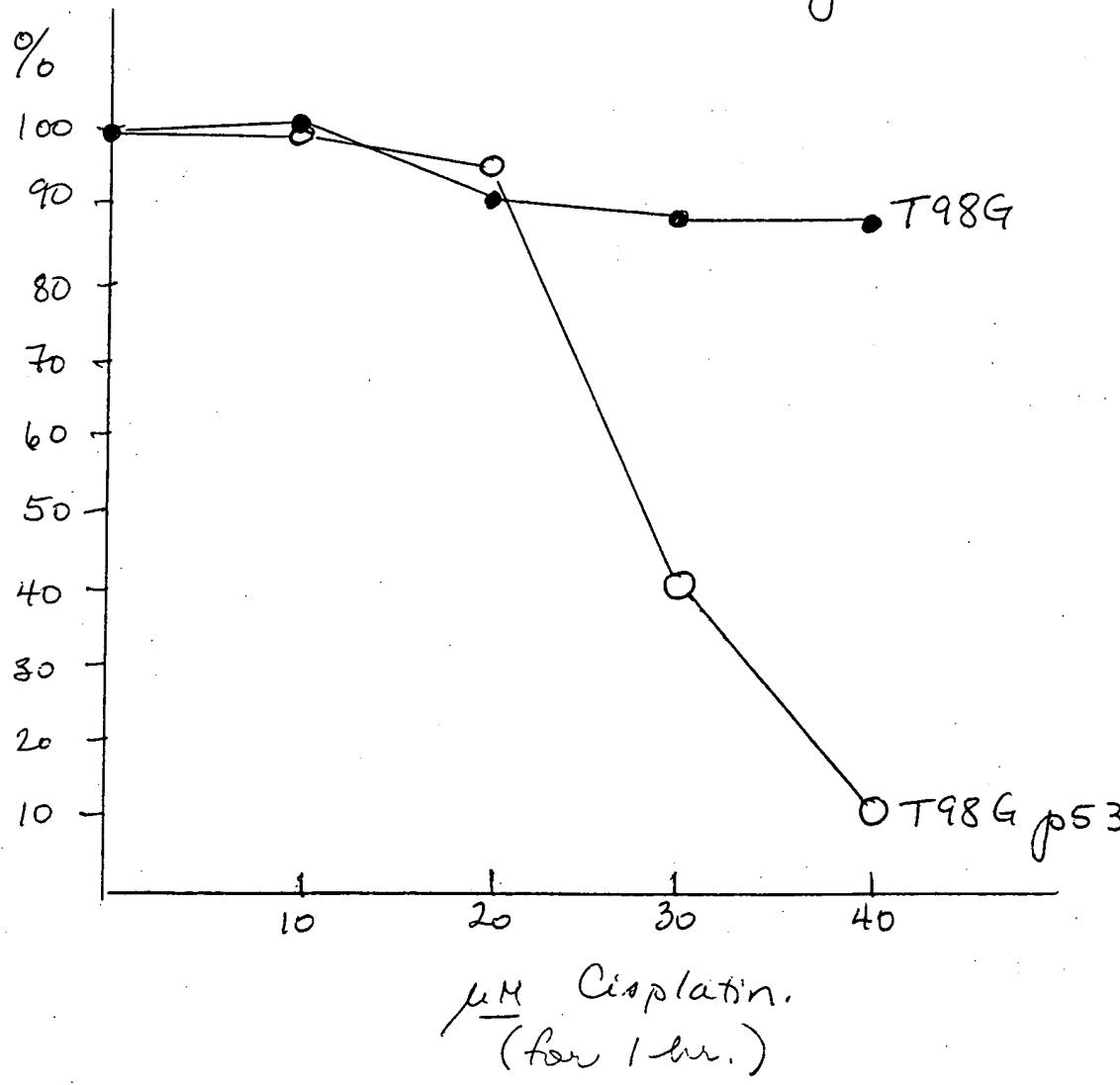
EC 50 $\sim 50 \mu\text{M}$ T98G.

Cells treated 1 hr. with varying doses of cisplatin,
trypsinized and settled in some wells; counted
one week later.



Cisplatin sensitivity of T98G cells.
T98G p53

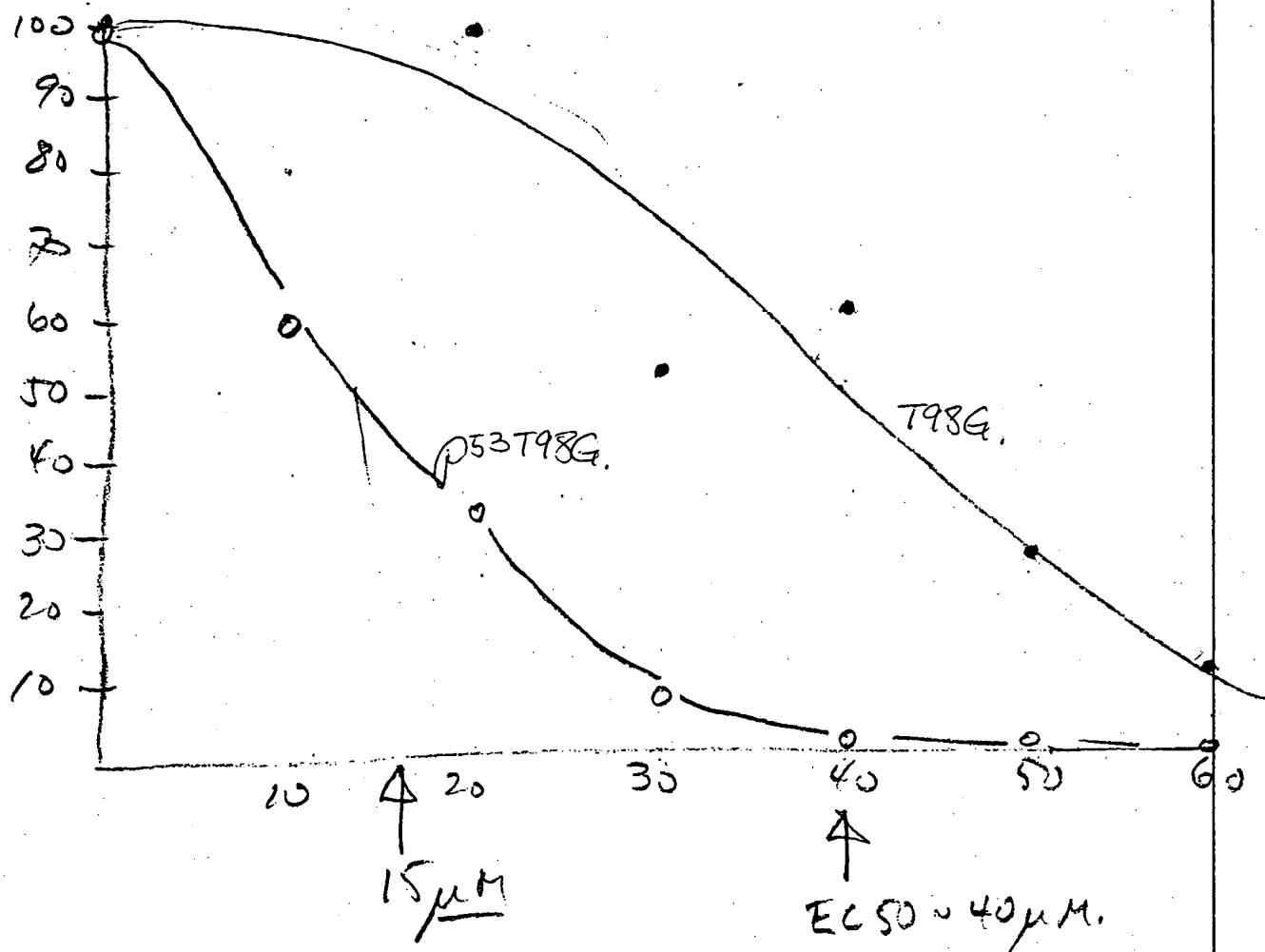
% maximum cell growth at 7 days



O I P E
JAN 07 2003

Jason's repeat of the Cisplatin
Sensitivity experiment.

50 SHEETS EYE-EASE 5 SQUARE
100 SHEETS EYE-EASE 5 SQUARE
200 SHEETS EYE-EASE 5 SQUARE
100 RECYCLED WHITE 5 SQUARE
42-381 42-382 42-389 42-392 42-393
National® Brand





PS 3
T9K6

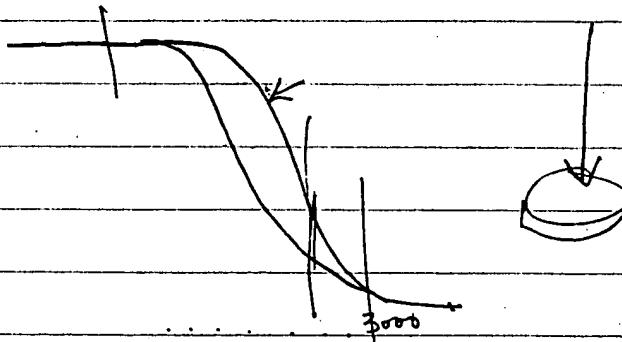
T986

O A	$107/9$	2	12.3×10^4	11.5	100%	50	55	64	45	50	53.5	54	49.3
O B	$93/9$	2	10.7×10^4	10.7	100%	50	52	46	46	50	54		
10 C	$63/9$	2	6.7	58%	$57/9$	42						78%	85%
20 D	$42/9$	2	4.3	37		55	55	56	56	54	51.3	100%	110
30 E	$9/9$	2	1.0	9		26	31	32	32	29	32	23.2	54%
40 F	$1/9$	2	0.11	1		26	31	30	30	34	33		61%
50 G	$1/9$		0.11	1		18	20	20	12	17.5	18		83%
60 H	$1/9$		0.05	0.5%		14	16	14	10	13.5	14		26%

OIP Radiation Experiment

JAN 07 2003

PATENT & TRADEMARK OFFICE
U.S.A.



1000 rads ~~time~~

500 rads 25 "

~~50 rads~~ ~~3 "~~

3 "

60 ~~60~~ 3 "

80 100 5 "

100 200 10 "

140 300 15 "

180 400 20 "

220 500 25 "

260 600 30 "

300 700 35 "

340 800 40 "

900 45 "

1000 50 "

1500 75 "

~~1200~~ 25
~~50,000~~ ~~T25~~
~~10,000~~.

12 flasks each line.

~~2000~~
T98G

3 series of 12 T25 flasks were set

up on

(1) T98G parental line

(2) T98G p53 propagated in E418 until
time of plating

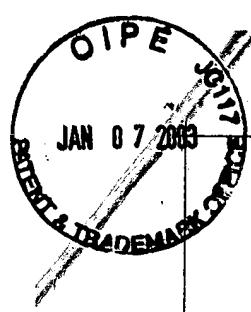
(3) T98G p53 propagated two weeks
10/10 E418.

Cells were allowed to attach for 24 hrs
prior to irradiation.

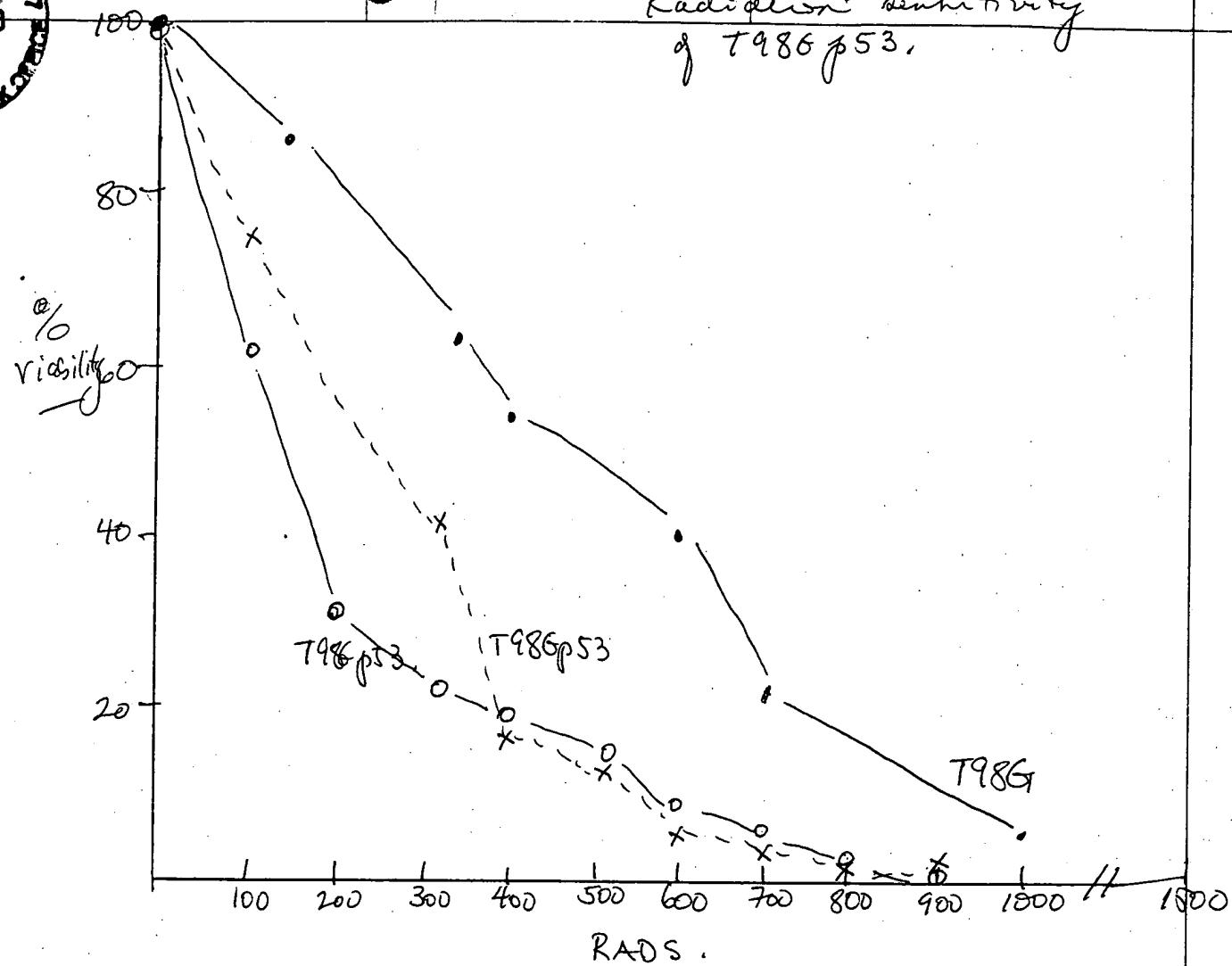


JAN 07 2003

EFFICIENCY LINE #22-206



Radiation sensitivity
of T986 p53.



Colonies of 1986 counted at 5 deg.

Colonies of T986 p53 counted at 12 days.

JAN 07 2003
PATENT & TRADEMARK OFFICE

Reoeration Sensitivity

PERCENT SURVIVAL

